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ECO-FRIENDLY MANAGEMENT OF DAMPING OFF (*PYTHIUM APHANIDERMATUM*) OF CHILLI (*CAPSICUM ANNUM* .L)

ATTAULLAH HANIF, ABHILASHA A. LAL & SOBITA SIMON

Department of Plant Pathology, SHIATS, Allahabad, Uttar Pradesh, India

ABSTRACT

Chilli is often found affected by damping off disease in nursery stage. Owing to the great loss of the seedling an investigation was undertaken to manage the damping off disease of chilli. The experiment was conducted under *in vitro* and pot culture conditions to observe the effect of bio-agents, botanicals and fungicide against *Pythiumaphanidermatum*. Five treatments were taken up with four replications and data collected was analyzed using CRD. Maximum inhibition percent was observed in *Trichoderma viride* (71%) followed by *Trichodermaharzianum* (63.73%), *Pseudomonas fluorescens* (61.66%), carbendazim (100%) as compared to control (0%). *T. viride* was significantly superior as compared to other treatments. In pot condition six treatments were taken up with four replications and data collected was analyzed using RBD. The treated seeds were sown in pathogen inoculated soil @100 seeds per pot and irrigated daily. Pathogen alone inoculated pots served as control. The observation was recorded on 14 days after sowing. The results revealed that seed treatment with the fungicides and bio-agents against damping off (*Pythiumaphanidermatum*) as compared to check were significant. Maximum germination percent was recorded in *T.viride* (83.75%) followed by *T. harzianum* (66.75%), *P.fluorescens* (65%), neem cake (56%), neem oil (43%) and carbendazim (78%) as compared to control (27%). *Trichoderma viride* was superior as compared to other treatments.

KEYWORDS: Bio-Agents, Botanicals, Chilli, Fungicides, *Pythiumaphanidermatum*

INTRODUCTION

Chilli is known from pre-historic times in Peru. They are believed to have originated in the tropical America. It is also said that chilli has originated in the Latin American regions of the New Mexico and Guatemala as a wild crop around 7500BC, as per the remains of the pre historic Peru. The people native to these places domesticated this crop in and around 5000 BC. Chilli is said to be the first ever domesticated crop in America. The three species *Capsicum annuum, C. frutescens* and *C. chinese* evolved from a common ancestor located in the North of the Amazon basin (NW-Brazil, Columbia) **(Tiwari, 2010).** *Pythium*species are essentially soil borne and possesses a great problem in disease management **(Shiekh, 2010).** Damping-off disease is difficult to control by small component of action. Usually the fungus survives in soil in absence of suitable environment and host for infection. As the fungus causes various types of diseases of different plant species, isolation of fungus from diseased tissue and detection of fungus from soil becomes an important aspect for development of suitable management strategies (**Yadav and Joshi, 2012).**

The use of bio-agents and botanicals for plant protection has assumed greater importance in recent years all over the world due to environmental pollution and health hazards associated with the indiscriminate use of synthetic fungicides, use of fungicides can be minimized by the integrated approach towards the management of plant diseases. In this study we

aim to find the safe and cheap way to manage the damping off of chilli and to provide good and economically accessible method of disease control with bio-agents and botanicals.

MATERIALS AND METHODS

Colony Growth Inhibition assay with *Trichoderma* spp. and *Pseudomonas fluorescens* in dual Culture Method

Firstly, the antagonistic activity of *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* against *Pythiumaphanidermatum* was studied in dual culture method **(Falck, 190**7). So the antagonist was evaluated by dual culture technique. The pathogen was inoculated on one side of the petri plate filled with 20 ml of PDA and antagonist was inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing five days old culture was used. In case of bacterial antagonist evaluation, bacterial antagonist was streaked in the plates and fungal discs were placed at one corner of the plates. After a period of incubation, when the growth of the pathogen was measured at 48, 72, 96 and 120 hrs. Percent inhibition over check was worked out according to the equation given by **(Vincent, 1927).**

Radial growth (mm) was recorded at 48, 72, 96 and 120 hrs. (Figure 1 and 2). For the evaluation and knowing the effect of carbendazim against *Pythiumaphanidermatum* poison food technique was used (Table 1). A total of seven treatments with five replications were analyzed using CRD.

Morphological Identification and Characteristics of *Pythiumaphanidermatum*

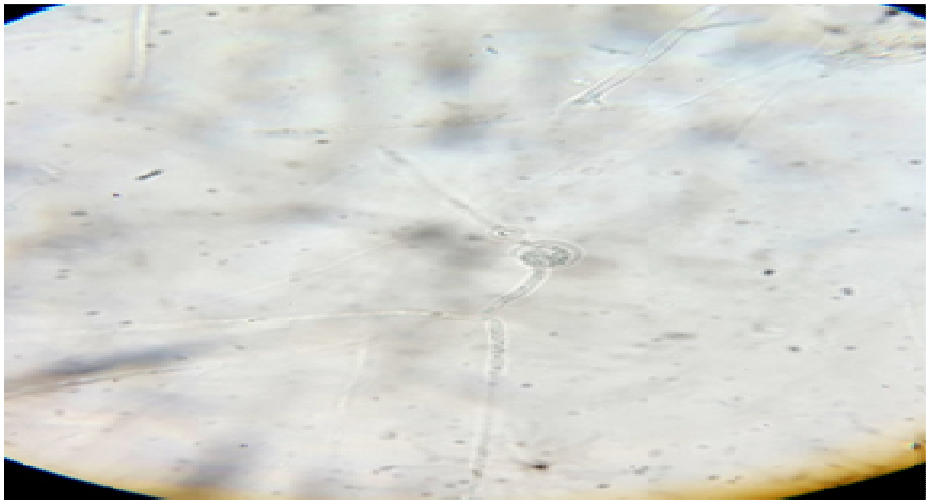

Figure 1: Microscopic View of *Pythiumaphanidermatum* (40x)

The slides were made from the culture of isolated pathogen and also directly from the infected portion of the toppled seedling and was observed under simple microscope (Figure 1). The sporangia, mycelia and other characteristic features were compared with standard book for identification. The main and traditional methods for identifying fungal species are based on morphological and physiological studies. Identification of *Pythiumaphanidermatum* was based on standard keys suggested by (**Plaats- Niterink, 1981; Butler, 1907 and Dick, 1990)**. Slides were prepared from the culture and stained with cotton blue according to (**Parija and Prabhakar, 1995)** and examined under the light microscope**.** The mycelium presents a white, fluffy appearance and consists of long, slender hyaline much branched hypha. Usually there is a slight constriction at the base of side branch. The hyphae are generally regular in diameter but taper evenly towards their tips (Figure 1)**.**

Preparation of Fungal Inoculum

A pure culture of *Pythiumaphanidermatum* was isolated from infected seedlings. Inoculum of the funguswas prepared on sorghum grains. For inoculation, a known weight of sorghum seeds colonized with the fungus was grounded and used with distilled water and standardized to 2 g seeds/10 ml (1 ×106 cfu/gseed) **(Khan and Haque, 2012).**

RESULTS AND DISCUSSIONS

The data on the mycelial growth (mm) was influenced by bio-agents and fungicide and are given in the (Table 1).

Figure 2: Mycelial Growth Inhibition of *Pythiumaphanidermatum* at 120 Hours as Affected by Bio-Agents and Carbendazim

At 48, 72, 96 and 120 hrs after inoculation of the pathogen (*Pythiumaphanidermatum*) on PDA medium significant differences in mycelial growth were observed among the treatments. Minimum mycelial growth of the test pathogen was recorded with *T. viride*. All treatments were statistically significant as compared to untreated check but among the treatments (*Trichoderma viride* and *T. harzianum*), (carbendazim and *Pseudomonas fluorescens*) are non-significant (Figure 1).

It was observed that the growth of *Pythiumaphanidermatum* was inhibited by bio-agents and treated check. Maximum inhibition at 120 hours after inoculation was observed with *T. viride* (71%) among the bio-agents whereas, the treated check *i.e.*carbendazim recorded 100% and the untreated check recorded no inhibition.

Table 1: Mycelial Growth (mm) and Inhibition Per Cent of *Pythiumaphanidermatum*as Affected by Different Treatments

Treatments	Radial Growth (mm) of *Pythiumaphanidermatum*				Per Cent Inhibition at 120 hrs
	48 hrs	72 hrs	96 hrs	120 hrs	
Untreated check	4.35	8.37	8. 75	9.65	0
Treated check (Carbendazim)	0	0	0	0	100
Trichodermaviride	2.3	2.35	2.5	2.75	71.50
Trichodermaharzianum	2.5	3	3	3.5	63.73
Pseudomonas fluorescens	2.5	2.5	2.75	3.7	61.66
S.Ed. (±)	**0.385**	**0.108**	**0.443**	**0.147**	**0.147**
C.D.(P= 0.05)	**1.601**	**0.317**	**1.729**	**0.992**	**0.992**

However, treated check (carbendazim) is found significantly different among all treatments *Pseudomonas fluorescens*;*Trichodermaviride*and *Trichodermaharzianum*were found significant among themselves.

A significant difference in inhibition percent of mycelial growth was observed among the treatments. Maximum

percent inhibition was recorded in *Trichodermaviride* (71.50%) whereas it was 100% in carbendazim. All treatments were statistically significant but among the treatments *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and carbendazim were non-significant.

The results presented in table 1 showed that tested bio-agents exhibited antagonistic activities against*Pythiumaphanidermatum.* Radial growth of the pathogen was considerably hindered by all the test antagonists under the condition of this study. *Trichodermaviride* was most antagonistic and inhibited the radial growth of *P.aphanidermatum* as they have more than one mechanism of action.

Table 2: Per Cent Germination of Chilli at 14 and 28 DAS as Affected by Different Treatments

Treatments		14 DAS	28 DAS
1	Untreated check	22.25	27.25
2	*Trichodermaviride*	77.25	83.75
3	*T. harzianum*	61.75	66.75
4	*P. fluorescens*	60.00	65.50
5	Neem cake	49.50	56.25
6	Neem oil	38.75	43.00
7	Treated check (Carbendazim)	67.50	78.50
F- test		**S**	**S**
S. Ed. (±)		3.20	3.25
C. D. (P = 0.05)		**6.691**	**6.839**

The data (Table 2) showed significant effect of bio agents, neem cake and fungicide on germination of chilli at 14 and 28 DAS. The results revealed that there was significant increase in the germination percentage of chilli in*Trichodermaviride* (83.75), treated check (carbendazim) (78.50), neem cake (56.25), *Pseudomonas flourescens* (65.50), *T. harzianum* (66.75), neem oil (43.00) as compared with the untreated check (27.25). However, *Trichodermaviride*and treated check (carbendazim) are found significant and also*Pseudomonas flourescens*, *T. harzianum*were found significant among themselves.

Hence, there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals, bio- control agents work best when pathogen pressure is low to moderate, because their activities against pathogens are biological by nature; it is possible that they will not be effective when overwhelmed by high pathogen levels. In addition, bio- control agents are generally not effective if once the plants have been infected by *Pythium* and thus should not be considered curative control treatments **(Kumar and Mukherjee, 1996 and Khare*et al.*, 2010).**

CONCLUSIONS

Trichodermaviride @ 4g/kg seeds proved its efficacy as an antagonist under *in vitro* and pot culture conditions against *Pythiumaphanidermatum*causing Damping off of chilli. Although use of synthetic chemicals is more effective than *T. viride* but they also have adverse effect on environment. However, the present research findings are limited to one crop season under Allahabad agro-climatic condition as such more trials are required in future to validate the findings.

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